Journal Pre-proofs

Immediate and long-term effects of mechanical loading on Achilles tendon volume: A systematic review and meta-analysis

Eman Merza, Stephen Pearson, Glen Lichtwark, Meg Ollason, Peter Malliaras

PII: S0021-9290(21)00069-5
DOI: https://doi.org/10.1016/j.jbiomech.2021.110289
Reference: BM 110289

To appear in: *Journal of Biomechanics*

Received Date: 29 May 2020
Revised Date: 14 January 2021
Accepted Date: 23 January 2021

Please cite this article as: E. Merza, S. Pearson, G. Lichtwark, M. Ollason, P. Malliaras, Immediate and long-term effects of mechanical loading on Achilles tendon volume: A systematic review and meta-analysis, *Journal of Biomechanics* (2021), doi: https://doi.org/10.1016/j.jbiomech.2021.110289

Title page

Title:

Immediate and long-term effects of mechanical loading on Achilles tendon volume: A systematic review and meta-analysis

Authors:

Eman Merza [a*], Stephen Pearson [b], Glen Lichtwark [c], Meg Ollason [d], Peter Malliaras [a].

[a] *Department of physiotherapy, Faculty of Medicine, Nursing and Health Science, Monash University, Frankston Vic 3199, Melbourne, Australia,* Email: iman.merza@monash.edu

[b] *Centre for Health, Sport and Rehabilitation Sciences Research, University of Salford, Greater Manchester, M5 4WT, United Kingdom,* Email: s.pearson@salford.ac.uk

[c] *Centre for Sensorimotor Performance, School of Human Movement and Nutrition Sciences, The University of Queensland, St. Lucia, QLD 4072, Brisbane, Australia, Email:* g.lichtwark@uq.edu.au

[d] *Department of physiology, Biomedicine Discovery Institute, Monash University, Clayton, Vic 3800 Melbourne, Australia,* Email: moll1@student.monash.edu

[a] *Department of physiotherapy, Faculty of Medicine, Nursing and Health Science, Monash University, Frankston Vic 3199, Melbourne, Australia*, Email: peter.malliaras@monash.edu

[*] Corresponding Author:

Eman Merza, Email: iman.merza@monash.edu

Phone Number: +61426896790

Word count:

Abstract: 247

Main Text: 3,938

Abstract

The Achilles tendon (AT) may experience changes in dimensions related to fluid flow under load. The extent to which fluid flow involves redistribution within or flow out of the tendon is not known and could be determined by investigating volume changes. This study aimed to synthesize data on immediate and long-term effects of loading on tendon volume among people with a healthy AT and midportion Achilles tendinopathy (MAT). A secondary aim was to synthesise data from the included studies investigating parallel change in cross-sectional area and length. Systematic electronic search was performed in MEDLINE, EMBASE, CINAHL, AMED, and Scopus from inception until May 2020. Standardized mean differences (SMDs) were calculated for intervention-induced changes from baseline for all outcomes. Methodological quality was assessed using modified version of Newcastle Ottawa Scale (NOS). Twelve studies were included in meta-analysis. For healthy AT, there were negligible to small changes in volume following cross-country running (-0.33 [95% CI=-1.11 to 0.45] (*P*=0.41)) and isometric exercise (0.01 [95% CI= -0.54 to 0.55] (*P*=0.98)) and a large increase at the short-term with 12-week isometric protocol (0.88 [95% CI= -0.10 to1.86] (*P*=0.08)). For MAT, there was an immediate large reduction in volume with isometric exercise (-1.24 [95% CI= -1.93 to -0.55] (*P*=0.0004)), small increase with eccentric exercise (0.41 [95% CI= -0.18 to 1.01](*P*=0.18)) and small reduction at the short-term with long-term interventions (-0.46 [95% CI= -0.87 to -0.05] (*P*=0.03)). This meta-analysis suggests that healthy AT remain isovolumetric with acute interventions while MAT exhibit immediate and short-term volume reductions in response to different interventions.

Keywords: Achilles tendon, Tendon volume, Fluid flow, Mechanical load.

Abbreviations:

AT: Achilles Tendon

MAT: Midportion Achilles Tendinopathy

CSA: Cross Sectional Area

3DUS: Three dimensional Ultrasound

MRI: Magnetic Resonance Imaging

1. Introduction

The Achilles tendon (AT) is a compliant structure that exhibits viscoelastic behavior when mechanically loaded (Butler, 1997, Kjær, 2004, Han et al., 2000). The AT is composed of collagen fibers that are organized in a hierarchical pattern and immersed within a hydrophilic extracellular matrix (Kjær, 2004, Screen et al., 2015). This unique structure promotes elasticity and allows the AT to tolerate substantial forces during load-bearing activities (Finni et al., 2000, Lichtwark and Wilson, 2005). Repetitive loading of the AT during high impact physical activities (i.e. running and jumping) may lead to injury (referred to as tendinopathy) if the potential of the tendon to adapt to load is exceeded (Magnusson and Kjaer, 2019). Midportion Achilles tendinopathy (MAT) is a common sport-related disorder characterized by often long-lasting localized tendon pain and disability in tendon loading activities. Although MAT is often managed with progressive tendon loading over several weeks (Malliaras et al., 2013), the evidence shows limited tissue recovery and adaptation (Drew et al., 2014, Farnqvist et al., 2019).

Tendon is a viscoelastic tissue meaning that adaptation is time-dependent (Pearson et al., 2007). Tendon viscoelasticity results from the interaction between tendon fluid and solid matrix components (Wall et al., 2016, Docking, 2013, Kjær, 2004). It is known that there is transient fluid movement (fluid flow) with loading and this may influence matrix remodeling and tendon adaptation at a cellular level (Butler, 1997, Hannafin, 1994, Han et al., 2000). In vitro studies show that static and cyclic tensile loading induced reduction in fluid content due to fluid flow from the tendon core to the periphery (exudation) which was associated with multiple metabolic and biochemical reactions (i.e. release of inflammatory signals, synthesis of matrix proteins, and inhibition of collagenase mRNA) (Butler, 1997, Wall et al., 2016, Docking, 2013, Hannafin, 1994, Lavagnino et al., 2003).

There is also a relationship between the amount of tendon fluid and the viscoelastic properties within a tendon (Atkinson et al., 1997, Haut, 1997). In this regard, Haut (1997) found that sufficiently hydrated tendons display greater stiffening under high tensile loading rates compared to tendons with less fluid content. This mechanical response that is referred to as hydraulic stiffening is known to provide protection from load-induced damage in viscoelastic tissues (Haut, 1997, Kafka, 1993). However, repetitive loading may cause transient fluid flow that would reduce hydraulic stiffening and increase tissue strain (Butler, 1997, Hannafin, 1994, Lozano et al., 2019, Haut, 1997). This may occur in certain regions of the tendon in the case of fluid redistribution within the tendon, or throughout the tendon if there is exudation.

One recent review identified evidence for a transient reduction in tendon cross-sectional area (CSA) and stiffness in response to acute loading in humans (Obst et al., 2013). However, at the time of this review, there was limited evidence for adaptation in volume immediately following loading and only one included study investigated this outcome (Shalabi et al., 2004a). This is a limitation because CSA change may indicate transient fluid redistribution as opposed to fluid exudation. The distinction is important because tendon mechanical effects of each may be different. With redistribution, it may be possible that some portions of the tendon become stiffer (those with more fluid) and others less stiff (those with less fluid), exposing the less hydrated regions to injury.

In contrast to the immediate response, there is evidence from a systematic review that tendon CSA increases in response to chronic load in humans (Wiesinger et al., 2015). An increase in CSA is likely related to changes in fibril diameter, fibril density, collagen, and proteoglycans content (Magnusson and Kjaer, 2003, Magnusson and Kjaer, 2019). It is plausible that frequent episodes of transient fluid flow in response to loading stimulates matrix remodeling

and eventually leads to an increase in CSA. Whether volume changes in parallel to CSA is not clear. The difference with volume is that it indicates whether adaptation is occurring globally in the tendon or in specific regions.

Given the key role of tendon fluid in determining tendon mechanics, it seems important to understand how tendon volume may change immediately after loading and to long-term loading. This morphological adaptation might be altered in tendinopathy. Looking at volume may improve our understanding of how tendons (healthy vs pathological) adapt morphologically to acute and long-term loading. Therefore, this study aimed to synthesize data on the immediate and long-term effects of mechanical loading on free AT volume among people with healthy tendons and MAT. Within-group changes in volume, as well as between-group comparisons in volume change between symptomatic and asymptomatic AT, were considered. A secondary aim was to synthesize data from the included studies investigating the parallel change in CSA and tendon length.

2. **Methods**

2.1 Data sources and search

The review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Moher et al., 2009) and was registered with the International Prospective Register of Systematic Reviews (PROSPERO; reference CRD42020146733). Two reviewers (E.M, M.O) performed an independent literature search for studies examining the effect of mechanical load on AT volume among healthy people or people with MAT. Searches were conducted in the following databases: MEDLINE (Ovid), EMBASE (Ovid), CINAHL (via EBSCOHost), AMED (Ovid), Scopus and Google scholar

from inception to May 2020. Gray literature was also searched via OpenGray using the terms 'Achilles tendinopathy' [condition] and 'mechanical load' [intervention] in September 2019. The search strategies for all databases are shown in Appendix 1.

2.2 Criteria for considering studies for this review

2.2.1. Types of studies

Any study design that allowed prospective investigation of change in AT volume within the cohort of interest (healthy or MAT) or comparison of change between these cohorts were considered. This included randomised trials, cohort studies (single cohort and cohort studies that included healthy and control participants), and case series. Case studies were excluded.

2.2.2. Types of participants

Studies that included healthy or MAT participants (or both) were considered. Healthy participants had to be free of current or prior Achilles tendon pain or pathology. We accepted any study that labelled participants as having MAT.

2.2.3. Types of interventions

Studies including any type of mechanical loading (i.e. acute bout of loading or long-term interventions) including isotonic, isometric, stretching, and ballistic type loading such as hopping, running, and jumping. There was no minimum intensity or volume of loading.

2.2.4. Type of outcomes

Studies that investigated the change in AT volume pre and immediately post-intervention or during contraction were considered. Data for change in volume over other timepoints was also extracted. Tendon volume could be measured by either freehand 3-dimensional

ultrasound (3DUS) or magnetic resonance imaging (MRI). Studies that measured volume from the calcaneal insertion of the tendon and at least up to the soleus muscle-tendon junctions or higher were included. Although it was not a selection criterion, tendon CSA and length were extracted if reported. The outcome times of interest were immediate changes (immediately post-loading within 10 minutes or during contraction) and changes beyond this period (defined as short-term up to 6 months and long-term >6 months).

2.3. Selection of studies

The two reviewers independently screened studies in the search yield. Titles and abstracts were initially screened based on eligibility criteria, followed by a full-text screen of studies that appeared to be eligible or eligibility could not be determined after the title and abstract screen. The reference lists of included studies were manually checked for any relevant studies. Forward searches were performed in Google Scholar for studies that had cited studies that were included. Discrepancies between the two reviewers at any stage were resolved via discussion or by consulting a third reviewer (P.M).

2.4. Data extraction

To minimize bias, two reviewers separately extracted data from the included studies using a piloted data extraction form. Data extracted included the study design, sample size, description of participants (demographic, injury data such as duration and severity), exercise intervention (contraction type, sets, repetitions or hold time, rest times, intensity), comparison intervention (if applicable), outcome measure (s) of interest [as described above] and measurement methods, region of interest in the tendon (free AT, proximal or whole tendon) and pre- and post-intervention data for all outcome measures.

2.5. Methodological quality assessment

The two reviewers independently assessed the methodological quality of the included studies using a modified version of the Newcastle Ottawa Scale (NOS). This version has been developed to assess the methodological quality for any observational study design and has been used in previous systematic reviews (Bawor et al., 2015, Martinez-Calderon et al., 2018). The tool includes four main domains of quality assessment including selection bias, performance bias, detection bias, and information bias (Martinez-Calderon et al., 2018). The four domains include a total of seven items and agreed decision rules were developed for each item (table. 2). The reviewers graded each item as definitely yes=3, mostly yes=2, mostly no= 1, and definitely no=0 according to the provided information in each article. Therefore, the maximum score for each study would be 21 points. Any disagreement between reviewers was discussed and if needed the third reviewer (P.M) was consulted.

2.6. Measures of treatment effect

Review Manager [(**RevMan**) [Computer program]. Version **5.3**. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, (2014)], was used to calculate measures of treatment effect. Standardised mean difference (SMD) and 95% confidence intervals (CI) were calculated for continuous outcomes that were measured on different scales or mean difference (MD) if the scale was the same. The SMD of 0.2 represents a "small" effect, an SMD of 0.5 represents a "moderate" effect, and an SMD of 0.8 represents a "large" effect (Cohen, 1988). For any missing data (means and standard deviations), study authors were contacted via email twice within one month. Where no reply received from contacted authors, standard deviations (SD) were calculated from standard error (SE), 95% CI or *P*-value, or the median used to estimate the mean and SD calculated from the IQR range (SD=width of

IQR/35). In cases where the required data was not available qualitative synthesis was undertaken.

2.7. Data synthesis

Effect estimates from studies with similar characteristics (e.g. participants, outcomes [volume, CSA or length], and type of intervention) were pooled using Revman software. Within-group data were pooled separately for each group (healthy and MAT) and between groups comparison data (symptomatic and asymptomatic) if available. We pooled data from studies that measured changes in the outcomes either during a contraction or immediately following it because no change in volume is likely to occur between these timepoints. Data were also pooled for studies assessing short- and long-term effects due to chronic interventions.

A random-effects model was chosen a priori because clinical and methodological heterogeneity (some of which is unreported and unknown) is likely to exist. The Chi-square statistic (if $p<0.05$) and the I^2 statistic were utilised to assess statistical heterogeneity among studies (Deeks et al., 2011). Values of 0-40% for the I^2 statistic are considered not important, 20-60% represent moderate heterogeneity, 50-90% represent substantial heterogeneity and 75-100% represent considerable heterogeneity (Deeks et al., 2011).

3. Results

3.1. Search results

Electronic database search yielded a total of 3979 studies. After the removal of duplicates, the remaining 3707 articles were scanned based on titles and abstracts, and 12 potentially eligible studies were identified. After the full-text screening, one study was excluded after consultation with the third author (P.M), and one study was further included from manual searching of the reference lists of included studies (Kubo et al., 2012) making a final number of 12 included studies in the review. The search and study selection is outlined in fig. 1.

3.2. Participants and interventions characteristics

Table 1 shows the characteristics of participants, severity and duration of MAT symptoms, and details of interventions. There were 195 participants included in the review (sample size per study ranged from 6 to 25 participants). Seven studies included only male participants and the remaining included both genders (M: 58.2% and F: 41.8%). The mean (SD) age for participants ranged from 23 to 51 years. Participants in most included studies (8/12, 67%) were recreationally active (Grosse et al., 2016, Syha et al., 2014, Shalabi et al., 2004b, Tsehaie et al., 2017, Gardin et al., 2010, Kubo et al., 2012, Obst et al., 2014b, Obst et al., 2015) but many studies did not report activity data (Shalabi et al., 2004a, Nuri et al., 2018, Nuri et al., 2017a, Nuri et al., 2017b).

Among the 12 included studies, six were performed on healthy participants. Five of these were single cohort studies and one with a control group of matched untreated participants (Kubo et al., 2012). Interventions involved isometric (Nuri et al., 2017a, Obst et al., 2014b), eccentric (Obst et al., 2015), strenuous exercise including cross country running and rope skipping (Syha et al., 2014, Grosse et al., 2016), and long-term isometric (12 weeks) (Kubo et al., 2012). Volume (all studies), CSA, and length (3 studies each) were recorded immediately

following the intervention and in one study at the end of the 12-week isometric (Kubo et al., 2012).

Six studies included participants diagnosed with MAT. Two had included control groups of healthy matched controls (Nuri et al., 2018) and untreated MAT participants (Gardin et al., 2010). The remaining four were single cohort studies in which the comparator was the contralateral side (Shalabi et al., 2004b, Shalabi et al., 2004a, Tsehaie et al., 2017, Nuri et al., 2017b). One study(Nuri et al., 2017b) had included participants with unilateral MAT and confirmed the absence of pathology in the contralateral side on the basis of clinical examination. The three other studies had included participants with either bilateral or unilateral MAT and two had confirmed the presence or absence of pathology using MRI (Shalabi et al., 2004a, Shalabi et al., 2004b). Loading including isometric and eccentric and volume outcome was recorded at the immediate, short, or long term. The severity of MAT was assessed only in two studies (Nuri et al., 2018, Nuri et al., 2017b) using the Victorian Institute of Sport Assessment-Achilles questionnaire (VISA-A). The mean (SD) VISA-A for the two studies was 54.2 (16.5) [100-point scale, 100 is best] representing moderate to severe severity (Robinson et al., 2001).

3.3. The methodological quality of the included studies

Details of quality assessment are provided in table. 2. The scores for methodological quality ranged from 14 to 18, mean (SD) 15.8 (1.19) out of 21. For each item within the tool, the mean total scores ranged from 1.25 to 3. Most studies (66%) were downgraded for performance bias due to inadequate adjustment for confounders mean (SD) 1.25 (0.6). The statistical analysis method was not clearly presented in many studies (75%). However, most studies (91%) did not have any missing data and scored high for detection bias 2.9 (0.3). All

studies had objectively measured the primary outcome using valid and reliable assessment tools and had achieved full scores for items included in the information bias domain.

3.4. Effects of mechanical loading on tendon volume

The results of the present meta-analysis show that different mechanical loading seems to have negligible or small effect on healthy tendon volume. In contrast, pathological tendon volume reduced with both acute and long-term interventions.

3.4.1 Immediate effects of mechanical loading in healthy tendons

The pooled SMD shows a small reduction in AT volume (SMD= -0.33 [95% CI=-1.11 to 0.45] (*P*=0.41)) among two studies (n=13) investigating cross country running (Syha et al., 2014). There was negligible change in volume following 15 minutes of rope skipping (SMD= -0.15 [95% CI= -1.20 to 0.90] (*P*=0.78)) (Syha et al., 2014, Grosse et al., 2016) (figure-2). There was also negligible change in pooled estimate for volume (SMD= 0.01 [95% CI= -0.54 to 0.55] (*P*=0.98)) and length (SMD= 0.09 [95% CI= -0.46 to 0.63](*P*= 0.75)) (figure-5), and a moderate reduction in pooled CSA (SMD= -0.54 [95% CI= -1.10 to 0.02](*P*=0.06)) (figure-4) following isometric exercise among two studies (n=26) (Nuri et al., 2017a, Obst et al., 2014b). There was large reduction in CSA (SMD= -0.94 [95% CI= -1.73 to -0.16] (*P*=0.02)) and negligible change in length (SMD= 0.03 [95% CI= -0.71 to 0.77] (*P*=0.94)) following eccentric exercise (Obst et al., 2015) . Pre-loading volume data were missing from Obst et al. (2015) and it was excluded from meta-analysis for volume outcome.

3.4.2 Short-term effects of mechanical loading in healthy tendons

There was large increase in volume observed at the endpoint of a 12 week isometric protocol (SMD= 0.88 [95% CI= -0.10 to1.86] (*P*=0.08))(Kubo et al., 2012) (figure-2).

3.4.3 Immediate effects of mechanical loading in midportion tendinopathy

There was a large reduction in pooled SMD of AT volume (SMD= -1.24 [95% CI= -1.93 to -0.55] (*P*=0.0004)) (figure-3) and CSA (SMD= -1.01 [95% CI= -1.69 to -0.34] (*P*=0.003)) (figure-4) following acute isometric exercise among two studies (n= 30) (Nuri et al., 2018, Nuri et al., 2017b), but small changes in pooled length (SMD= 0.27 [95% CI= -0.36 to 0.89] (*P*=0.40)) (figure-5). There was small to moderate increase in volume following eccentric exercise (SMD= 0.41 [95% CI= -0.18 to 1.01](*P*=0.18))(Shalabi et al., 2004a).

3.4.4 Short-term effects of mechanical loading in midportion tendinopathy

There was small to moderate reduction in pooled SMD of AT volume (SMD= -0.46 [95% CI= -0.87 to -0.05] (*P*=0.03)) following long-term eccentric protocols in two studies (n= 50) (Shalabi et al., 2004b, Tsehaie et al., 2017) (figure-3).

3.4.5 Long-term effects of mechanical loading in midportion tendinopathy

There was a small change in volume (SMD= -0.15 [95% CI= -0.71 to 0.42] (*P*= 0.61)) at 4 years following a start point of 12-week eccentric protocol (Gardin et al., 2010) (figure-3).

3.4.6 Comparison of immediate and short-term change in AT volume among symptomatic and asymptomatic tendons

Acute isometric and eccentric exercises among three studies (total n=52) resulted in an immediate effect of volume (reduction) that favours symptomatic tendons (0.75 [95% CI= -0.23 to 1.74] (*P*=0.13)) (Nuri et al., 2018, Shalabi et al., 2004b, Nuri et al., 2017b) (figure-6). Likewise, the long-term interventions in two studies (n=50) resulted in a short-term effect of volume (reduction) that favours symptomatic tendons (0.43 [95% CI= -0.04 to 0.89] (p=0.07)) (Shalabi et al., 2004b, Tsehaie et al., 2017) (figure-6).

4. Discussion

This study synthesized data investigating the immediate, short- and long-term changes in AT volume in response to different loading interventions among people with healthy tendons and MAT. Findings suggest that healthy AT had no significant immediate changes in volume in response to different acute interventions and one study suggests an increase in volume in response to a 12-week intervention. In contrast, pathological tendons exhibit significant immediate and short-term volume reduction in response to different loading interventions. There was no evidence to support long-term change in MAT volume but only one study had investigated such long-term changes. The direct comparison in immediate and short-term volume change between symptomatic and contralateral asymptomatic tendons in response to acute and long-term interventions suggest a volume reduction that favors symptomatic tendons, however, there was a substantial heterogeneity I^2= 75% which may be explained by the different loading interventions (i.e. isometric vs eccentric). These findings should be interpreted in the context of study methodological quality. Most studies did not adequately control for confounders (e.g. age, gender, history, and type of physical activity) which suggests larger studies that control confounders are needed to confirm these findings.

4.1. Immediate load adaptation in healthy tendons

The findings of the present meta-analysis show a small or negligible change in healthy AT volume in response to strenuous ballistic activities (skipping rope or cross-country running) or isometric interventions. This was accompanied by a moderate (but not significant) reduction in CSA and negligible tendon elongation in response to isometric interventions (Nuri et al., 2017a, Obst et al., 2014b). It is plausible that the small sample sizes (n=6 and 7) in the studies investigating changes in volume with cross-country running did not provide sufficient statistical power to detect any significant volume change.

The present study shows that the free AT elongation was negligible in response to acute isometric and eccentric interventions. Similarly, a very small change (< 1%) in the free tendon length of healthy AT was observed following a 5 km running intervention (Lichtwark et al., 2013). Our findings also suggest a moderate but not significant reduction in CSA in response to submaximal isometric interventions (n= 26), while eccentric intervention from one study (n=14) (Obst et al., 2015) induced a large and significant CSA reduction. Inconsistent findings are reported in the literature for the effect of different loading interventions including static stretching (5-min) and running (20-min) on tendon CSA (Burgess et al., 2009, Neves et al., 2020, Chiu et al., 2016).

The isovolumetric behavior and the parallel reduction in CSA among healthy tendons in response to acute loading likely indicate fluid redistribution without fluid exudation. The ability of healthy AT to preserve fluid content with acute loading might be a positive response to reduce the amount of mechanical load placed on the solid matrix components and to create hydraulic stiffening. A transient stiffening with loading may provide larger resistance to tension and protect the tendon from strain-induced injury (Haut, 1997, Kafka, 1993). Indeed, in a fluid, the stresses which arise from shearing the fluid do not depend on the

distance the fluid has been sheared; rather, they depend on how quickly the shearing occurs (Pearson et al., 2007).

Beside the key role of strain load in stimulating cell signaling and maintaining tendon integrity (Wall et al., 2016, Benjamin, 2000), fluid redistribution in healthy AT may provide additional stimulation for tendon cells (i.e. tenocytes) via fluid flow-induced shear forces (Lozano et al., 2019, Butler, 1997, Hannafin, 1994, Archambault et al., 2001). Tenocytes are mechanosensitive and connect to each other via the dynamic gap junctions and via integrins connections to the matrix (Wall et al., 2016). Integrins and cytoskeleton tension provides a homeostatic set point for tenocytes that when altered in response to shear forces may stimulate multiple cellular activities that are essential for matrix remodeling (Wall et al., 2016, Archambault et al., 2001, Lavagnino et al., 2008, Docking, 2013). In this context, human investigations utilizing microdialysis technique have shown an immediate increase in metabolic and inflammatory activities in the peritendinous space of the AT in response to varied type of acute loading interventions (Langberg et al., 2002, Langberg et al., 1999a, Langberg et al., 1999b).

4.2. Immediate load adaptation in MAT

The present meta-analysis shows a large and significant volume reduction in response to acute isometric interventions (Nuri et al., 2018, Nuri et al., 2017b) which was coupled with a large and significant reduction in CSA and small tendon elongation. It is important to note that there was the opposite effect (increased volume) observed in one study investigating eccentric contractions (Shalabi et al., 2004a). Nevertheless, utilizing freehand 3DUS (Nuri et al., 2018, Nuri et al., 2017b) vs MRI (Shalabi et al., 2004a) to assess immediate volume changes with loading may explain the discrepancy in findings.

The large reduction in MAT volume in response to acute loading seems to occur as a result of the small increase in tendon length that might fail to offset the large and significant reduction in CSA (Nuri et al., 2018, Nuri et al., 2017b). This transient response might be attributable to the pathological course of tendinopathy. Particularly, the marked increase of large proteoglycans (PGs) (i.e. aggrecan and versican) and glycosaminoglycans in MAT causes excessive fluid content and greater positive pressure within the tendon core, which together may cause a larger amount of tendon fluid to exudate radially with loading (Parkinson et al., 2011, Scott et al., 2015, Butler, 1997, Wall et al., 2016, Han et al., 2000). Excessive fluid in MAT may shield pathological regions from further tissue insult. Therefore, reduction in fluid content with loading may expose these regions to a greater amount of external load, thereby, greater local and global strain and ultimately tissue damage. It is well documented in the literature that Achilles tendinopathy reduces the stiffness modulus and causes the tendon to strain more under a given force compared to unaffected tendons (Obst et al., 2018).

At the cellular level, reduction in fluid content and the greater resultant load-induced changes within pathological regions may activate a pro-inflammatory cellular response and perhaps exacerbate the degeneration process (Dakin et al., 2018). However, it is not known yet whether fluid exudation in MAT contribute to tendon pathology or is a positive stimulus for recovery.

4.3 Short- and long-term adaptation to load

There was a large but insignificant short-term increase in healthy tendons volume in response to 12-week isometric intervention (n=9)(Kubo et al., 2012). In contrast, there was a moderate and significant short-term volume reduction following 12- and 16-week eccentric protocol among pathological tendons (n=45) (Shalabi et al., 2004b, Tsehaie et al., 2017). It is plausible

that frequent episodes of acute fluid exudation during the training sessions may have reduced excessive fluid content associated with tendinopathy and hence reduced tendon volume in the long-term. However, the change in pathological tendon volume at 4 years following a 12-week eccentric protocol was negligible (n=24)(Gardin et al., 2010). The evidence on volume changes with long-term interventions is scarce in the literature and but inconsistent for tendon thickness which could be explained by varied type, intensity, and duration of interventions.

4.4 Magnetic resonance imaging versus freehand three-dimensional ultrasound

While both MRI and freehand 3DUS had demonstrated excellent reliability in measuring AT volume in humans (Obst et al., 2014a, Shalabi et al., 2005), methodological limitations to these imaging modalities may influence the volume estimates. For example, it might be difficult to differentiate between tendon CSA and the surrounding paratenon layer with ultrasound (Kruse et al., 2017). Given that the extruded fluid might accumulate within the space between the epitenon and paratenon layers (Wellen et al., 2005) inability to differentiate between tendon CSA and paratenon may mask volume changes. Although excellent soft-tissue contrast can be obtained with MRI (Khan et al., 2003), the partial volume effect (i.e. lack of contrast between adjacent tissues on the same voxel and therefore possessing a signal average of these tissues) may introduce error to volume measures (Ballester et al., 2002).

5. Limitations and Future research

The current meta-analysis has some limitations that should be considered. Only twelve studies were included from the search strategy and a few were included in each meta-analysis. These studies had small sample sizes (range from 6 to 25) which may have limited statistical power and the ability to identify a change in volume. The majority of studies

included recreationally active male participants (145 M: 41 F) which would limit the generalizability of the results to a different population. Given that habitual loading may alter tendon structure and may cause region-specific hypertrophy in the long-term, including recreationally active participants may have influenced observed tendon adaptive responses (Rosager et al., 2002, Magnusson and Kjaer, 2003). According to the inclusion criteria, only participants with MAT were considered, therefore the results cannot be extended to insertional tendinopathy or other pathologies including paratenonitis. Although indirect volume estimates could be obtained from available investigations that assessed CSA and length changes in response to loading using MRI (Arampatzis et al., 2010, Bohm et al., 2014, Shin et al., 2008), it is worth noting that the chosen slice thickness among some of these studies (relatively thicker compared to the included studies) may have affected sensitivity to detect CSA changes with loading.

6. Conclusion

The current study suggests that healthy AT may have distinct volume adaptation compared to MAT in response to different loading interventions. The healthy AT seems to remain isovolumetric in response to acute interventions, regardless of reductions in CSA as a result of fluid redistribution. In contrast, pathological tendons may display a significant reduction in volume in response to acute and long-term interventions.

Declarations of interest: none

Acknowledgments: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Archambault, J. M., Hart, D. A. & Herzog, W. 2001. Response of rabbit achilles tendon to chronic repetitive loading. *Connective Tissue Research,* 42**,** 13-23.

Atkinson, T. S., Haut, R. C. & Altiero, N. J. 1997. A Poroelastic Model That Predicts Some Phenomenological Responses of Ligaments and Tendons. *Journal of Biomechanical Engineering,* 119.

Ballester, M. A. G., Zisserman, A. P. & Brady, M. 2002. E stimation of the partial volume effect in MRI. *Medical Image Analysis* 6**,** 389–405.

Bawor, M., Dennis, B. B., Bhalerao, A., Plater, C., Worster, A., Varenbut, M., Daiter, J., Marsh, D. C., Desai, D., Steiner, M., Anglin, R., Pare, G., Thabane, L. & Samaan, Z. 2015. Sex differences in outcomes of methadone maintenance treatment for opioid use disorder: a systematic review and meta-analysis. *CMAJ Open,* 3**,** E344-51.

Benjamin, M. a. R. J. R. 2000. The Cell and Developmental Biology of Tendons and Ligaments. *Inlernanonal Review of Cytology,* 196.

Burgess, K. E., Graham-Smith, P. & Pearson, S. J. 2009. Effect of acute tensile loading on gender-specific tendon structural and mechanical properties. *Journal of Orthopaedic Research,* 27**,** 510-515.

Butler, S. L., Kohles, S.S.,Thieike, R.J. , Chen, C. , Vanderby Jr s, R. 1997. Interstitial fluid flow in tendons or ligaments: a porous medium finite element simulation. *Med. Biol. Eng. Comput,* 35**,** 742-746.

Chiu, T. C. R., Ngo, H. C., Lau, L. W., Leung, K. W., Lo, M. H., Yu, H. F. & Ying, M. 2016. An Investigation of the Immediate Effect of Static Stretching on the Morphology and Stiffness of Achilles Tendon in Dominant and Non-Dominant Legs. *PloS one,* 11.

Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences,* Hillsdale, New Jersey, Lawrence Erlbaum Associates: Routledge.

Dakin, S. G., Newton, J., Martinez, F. O., Hedley, R., Gwilym, S., Jones, N., Reid, H. A. B., Wood, S., Wells, G., Appleton, L., Wheway, K., Watkins, B. & Carr, A. J. 2018. Chronic inflammation is a feature of Achilles tendinopathy and rupture. *Br J Sports Med,* 52**,** 359-367.

Deeks, J. J., Julian PT., H. & Douglas G., A. 2011. Analysing data and undertaking meta-analyses. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration*

Docking, S. T., Samiric. Ebonie, Scase. Craig Purdam, Jill Cook 2013. Relationship between compressive loading and ECM changes in tendons. *Muscles, Ligaments and Tendons Journal* 3**,** 7-11.

Drew, B. T., Smith, T. O., Littlewood, C. & Sturrock, B. 2014. Do structural changes (eg, collagen/matrix) explain the response to therapeutic exercises in tendinopathy: a systematic review. *Br J Sports Med,* 48**,** 966-72.

Farnqvist, K., Malliaras, P. & Pearson, S. 2019. Adaptation of Tendon Structure and Function in Tendinopathy With Exercise and Its Relationship to Clinical Outcome. *J Sport Rehabil***,** 1-30.

Finni, T., Komi, P. V. & Lepola, V. 2000. In vivo human triceps surae and quadriceps femoris muscle function in a squat jump and counter movement jump. *Eur J Appl Physiol* 83**,** 416-426.

Gardin, A., Movin, T., Svensson, L. & Shalabi, A. 2010. The long-term clinical and MRI results following eccentric calf muscle training in chronic Achilles tendinosis. *Skeletal Radiology,* 39**,** 435-442.

Grosse, U., Syha, R., Gatidis, S., Grozinger, G., Martirosian, P., Partovi, S., Nikolaou, K., Robbin, M. R., Schick, F. & Springer, F. 2016. MR-based in vivo follow-up study of Achilles tendon volume and hydration state after ankle-loading activity. *Scandinavian Journal of Medicine & Science in Sports,* 26**,** 1200-1208.

Han, S., Gemmell, S. J., Helmer, K. G., Grigg, P., Wellen, J. W., Hoffman, A. H. & Sotak, C. H. 2000. Changes in ADC caused by tensile loading of rabbit achilles tendon: evidence for water transport. *J Magn Reson,* 144**,** 217-27.

Hannafin, J. A. a. A., Steven P. 1994. Effect of Cyclic and Static Tensile Loading on Water Content and Solute Diffusion in Canine Flexor Tendons: An In Vitro Study. *Journal of Orthopaedic Research,* 12.

Haut, T. L. H. a. R. C. 1997. The state of tissue hydration strain-rate-sensitive determines stiffness of human patellar tendon. *J. Bmnechanics,* 30**,** pp, 79-81.

Kafka, V. 1993. Letter to the editor on hydraulic strengthening of bones. *J Bmmechnnrs* 26**,** 761-762.

Khan, K. M., Forster, B. B., Robinson, J., Cheong, Y., Louis, L., Maclean, L., and & Taunton, J. E. 2003. Are ultrasound and magnetic resonance imaging of value in assessment of Achilles tendon disorders? A two year prospective study. *Br J Sports Med,* 37**,** 149–153.

Kjær, M. 2004. Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. *Physiological Reviews,* 84**,** 649-698.

Kruse, A., Stafilidis, S. & Tilp, M. 2017. Ultrasound and magnetic resonance imaging are not interchangeable to assess the Achilles tendon cross-sectional-area. *Eur J Appl Physiol,* 117**,** 73-82.

Kubo, K., Ikebukuro, T., Maki, A., Yata, H. & Tsunoda, N. 2012. Time course of changes in the human Achilles tendon properties and metabolism during training and detraining in vivo. *European Journal of Applied Physiology,* 112**,** 2679-2691.

Langberg, H., Olesen, J. L., Gemmer, C. & Kjaer, M. 2002. Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. *J Physiol,* 542**,** 985-90.

Langberg, H., Skovgaard, D., Karamouzis, M., Bulow, J. & Kjòr, M. 1999a. Metabolism and inflammatory mediators in the peritendinous space measured by microdialysis during intermittent isometric exercise in humans. *Journal of Physiology* 515**,** 919-927.

Langberg, H., Skovgaard, D., Petersen, L. J., Bulow, J. & Kjòr, M. 1999b. Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. *Journal of Physiology* 521**,** 299: 306.

Lavagnino, M., Arnoczky, S. P., Kepich, E., Caballero, O. & Haut, R. C. 2008. A finite element model predicts the mechanotransduction response of tendon cells to cyclic tensile loading. *Biomech Model Mechanobiol,* 7**,** 405-16.

Lavagnino, M., Arnoczky, S. P., Tian, T. & Vaupel, Z. 2003. Effect of amplitude and frequency of cyclic tensile strain on the inhibition of MMP-1 mRNA expression in tendon cells: an in vitro study. *Connect Tissue Res,* 44**,** 181-7.

Lichtwark, G. A., Cresswell, A. G. & Newsham-West, R. J. 2013. Effects of running on human Achilles tendon length-Tension properties in the free and gastrocnemius components. *Journal of Experimental Biology,* 216**,** 4388-4394.

Lichtwark, G. A. & Wilson, A. M. 2005. In vivo mechanical properties of the human Achilles tendon during one-legged hopping. *J Exp Biol,* 208**,** 4715-25.

Lozano, P. F., Scholze, M., Babian, C., Scheidt, H., Vielmuth, F., Waschke, J., Ondruschka, B. & Hammer, N. 2019. Water-content related alterations in macro and micro scale tendon biomechanics. *Sci Rep,* 9**,** 7887.

Magnusson, S. P. & Kjaer, M. 2003. Region-specific differences in Achilles tendon cross-sectional area in runners and non-runners. *European Journal of Applied Physiology,* 90**,** 549-553.

Magnusson, S. P. & Kjaer, M. 2019. The impact of loading, unloading, ageing and injury on the human tendon. *Journal of Physiology,* 597**,** 1283-1298.

Malliaras, P., Barton, C. J., Reeves, N. D. & Langberg, H. 2013. Achilles and patellar tendinopathy loading programmes : a systematic review comparing clinical outcomes and identifying potential mechanisms for effectiveness. *Sports medicine (Auckland, N.Z.),* 43**,** 267-286.

Martinez-Calderon, J., Meeus, M., Struyf, F., Miguel Morales-Asencio, J., Gijon-Nogueron, G. & Luque-Suarez, A. 2018. The role of psychological factors in the perpetuation of pain intensity and disability in people with chronic shoulder pain: a systematic review. *BMJ Open,* 8.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. & Group, P. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med,* 6**,** e1000097.

Neves, C. D., Sponbeck, J. K., Neves, K. A., Mitchell, U. H., Hunter, I. & Johnson, A. W. 2020. The Achilles Tendon Response to a Bout of Running is not affected by Triceps Surae Stretch Training in Runners. *Journal of Sports Science and Medicine* 19**,** 358-363.

Nuri, L., Obst, S. J., Newsham-West, R. & Barrett, R. S. 2017a. Regional three-dimensional deformation of human Achilles tendon during conditioning. *Scandinavian Journal of Medicine & Science in Sports,* 27**,** 1263-1272.

Nuri, L., Obst, S. J., Newsham-West, R. & Barrett, R. S. 2017b. The tendinopathic Achilles tendon does not remain iso-volumetric upon repeated loading: Insights from 3D ultrasound. *Journal of Experimental Biology,* 220**,** 3053-3061.

Nuri, L., Obst, S. J., Newsham-West, R. & Barrett, R. S. 2018. Three-dimensional morphology and volume of the free Achilles tendon at rest and under load in people with unilateral mid-portion Achilles tendinopathy. *Experimental Physiology,* 103**,** 358-369.

Obst, S. J., Barrett, R. S. & Newsham-West, R. 2013. Immediate effect of exercise on Achilles tendon properties: Systematic review. *Medicine and Science in Sports and Exercise,* 45**,** 1534-1544.

Obst, S. J., Heales, L. J., Schrader, B. L., Davis, S. A., Dodd, K. A., Holzberger, C. J., Beavis, L. B. & Barrett, R. S. 2018. Are the Mechanical or Material Properties of the Achilles and Patellar Tendons Altered in Tendinopathy? A Systematic Review with Meta-analysis. *Sports Medicine,* 48**,** 2179-2198.

Obst, S. J., Newsham-West, R. & Barrett, R. S. 2014a. InVivo measurement of human achilles tendon morphology using freehand 3-D ultrasound. *Ultrasound in Medicine and Biology,* 40**,** 62-70.

Obst, S. J., Newsham-West, R. & Barrett, R. S. 2015. Three-dimensional morphology and strain of the human Achilles free tendon immediately following eccentric heel drop exercise. *The Journal of experimental biology,* Part 24. 218**,** 3894-3900.

Obst, S. J., Renault, J. B., Newsham-West, R. & Barrett, R. S. 2014b. Three-dimensional deformation and transverse rotation of the human free Achilles tendon in vivo during isometric plantarflexion contraction. *Journal of Applied Physiology,* 116**,** 376-384.

Parkinson, J., Samiric, T., Ilic, M. Z., Cook, J. & Handley, C. J. 2011. Involvement of Proteoglycans in Tendinopathy. *J Musculoskelet Neuronal Interact* 11**,** 86-93.

Pearson, S. J., Burgess, K. & Onambele, G. N. 2007. Creep and the in vivo assessment of human patellar tendon mechanical properties. *Clin Biomech (Bristol, Avon),* 22**,** 712-7.

Robinson, J. M., Cook, J. L., Purdam, C., Visentini, P. J., Ross, J., MaVulli, N., Taunton, J. E. & Khan, K. M. 2001. The VISA-A questionnaire: a valid and reliable index of the clinical severity of Achilles tendinopathy. *Br J Sports Med* 35**,** 335–341.

Rosager, S., Aagaard, P., Dyhre-Poulsen, P., Neergaard, K., Kjaer, M. & Magnusson, S. P. 2002. Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. *Scandinavian journal of medicine & science in sports,* 12**,** 90-98.

Scott, A., Ludvig Backman & Speed, C. 2015. Tendinopathy – update on pathophysiology. *Journal of Orthopaedic & Sports Physical Therapy*.

Screen, H. R., Berk, D. E., Kadler, K. E., Ramirez, F. & Young, M. F. 2015. Tendon functional extracellular matrix. *J Orthop Res,* 33**,** 793-9.

Shalabi, A., Kristoffersen-Wiberg, M., Aspelin, P. & Movin, T. 2004a. Immediate achilles tendon response after strength training evaluated by MRI. *Medicine & Science in Sports & Exercise,* 36**,** 1841-1846.

Shalabi, A., Kristoffersen-Wilberg, M., Svensson, L., Aspelin, P. & Movin, T. 2004b. Eccentric training of the gastrocnemius-soleus complex ion chronic achilles tendinopathy results in decreased tendon volume and intratendinous signal as evaluated by MRI. *American Journal of Sports Medicine,* 32**,** 1286-1296.

Shalabi, A., Movin, T., Kristoffersen-Wiberg, M., Aspelin, P. & Svensson, L. 2005. Reliability in the assessment of tendon volume and intratendinous signal of the Achilles tendon on MRI: a methodological description. *Knee Surg Sports Traumatol Arthrosc,* 13**,** 492-8.

Syha, R., Springer, F., Grozinger, G., Wurslin, C., Ipach, I., Ketelsen, D., Schabel, C., Gebhard, H., Hein, T., Martirosian, P., Schick, F., Claussen, C. D. & Grosse, U. 2014. Short-term exercise-induced changes in hydration state of healthy achilles tendons can be visualized by effects of off-resonant radiofrequency saturation in a three-dimensional ultrashort echo time MRI sequence applied at 3 Tesla. *Journal of Magnetic Resonance Imaging,* 40**,** 1400-1407.

Tsehaie, J., Poot, D. H. J., Oei, E. H. G., Verhaar, J. A. N. & de Vos, R. J. 2017. Value of quantitative MRI parameters in predicting and evaluating clinical outcome in conservatively treated patients with chronic midportion Achilles tendinopathy: A prospective study. *Journal of Science & Medicine in Sport,* 20**,** 633-637.

Wall, M. E., Dyment, N. A., Bodle, J., Volmer, J., Loboa, E., Cederlund, A., Fox, A. M. & Banes, A. J. 2016. Cell Signaling in Tenocytes: Response to Load and Ligands in Health and Disease. *Adv Exp Med Biol,* 920**,** 79-95.

Wiesinger, H. P., Kosters, A., Muller, E. & Seynnes, O. R. 2015. Effects of Increased Loading on In Vivo Tendon Properties: A Systematic Review. *Med Sci Sports Exerc,* 47**,** 1885-95.

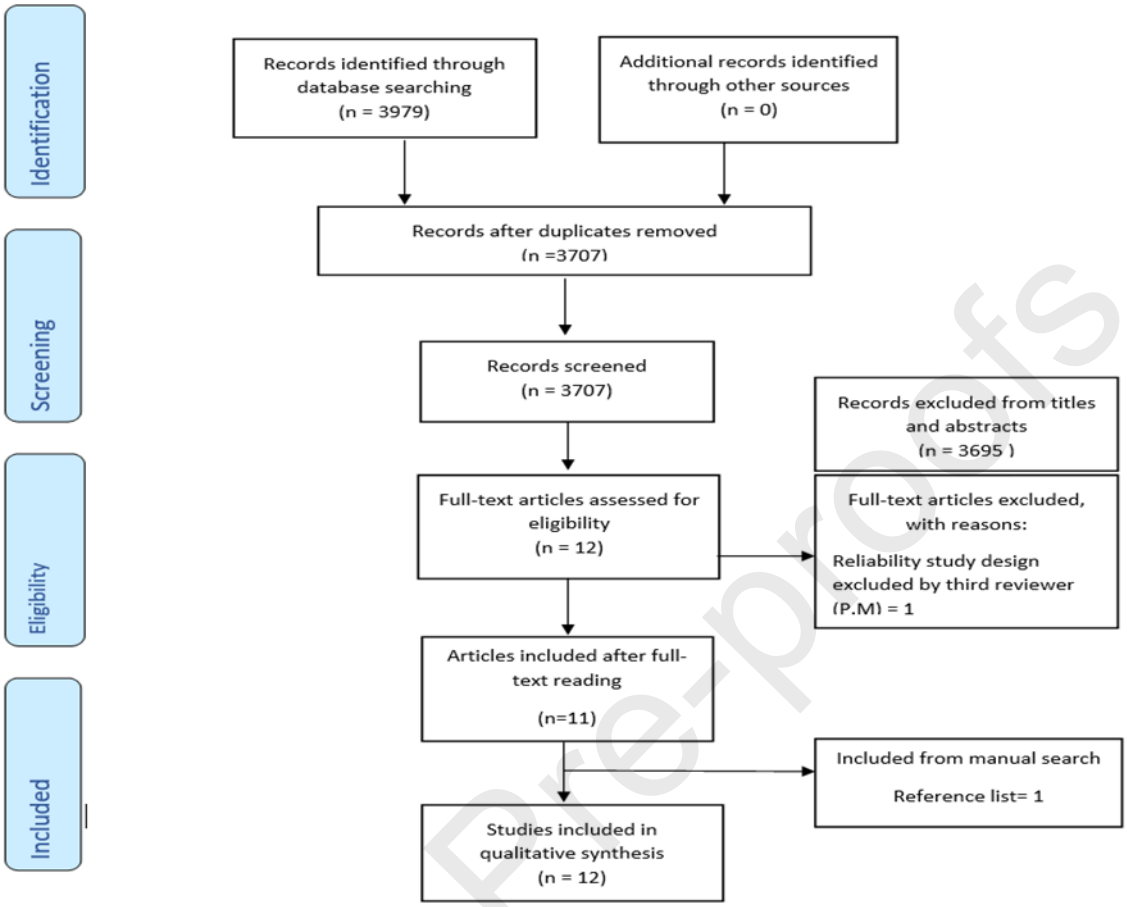

Fig. 1. PRISMA flow diagram

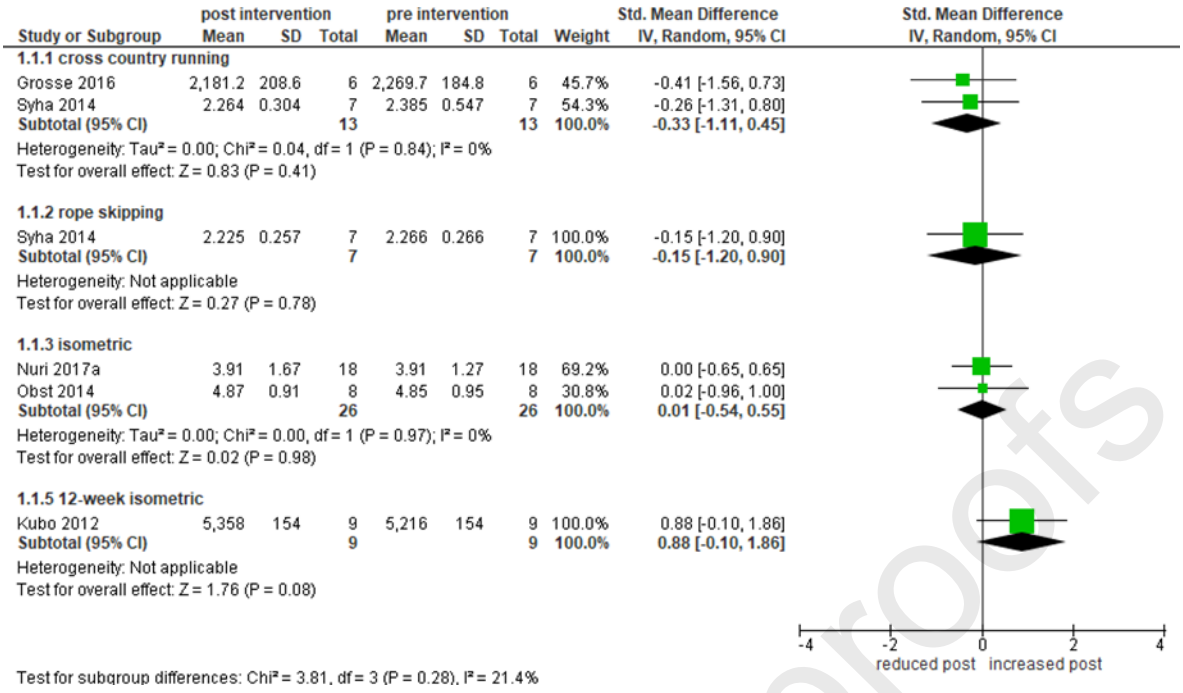

Fig. 2. Forest plot for the meta-analysis of the immediate and short-term effects of different interventions on healthy Achilles tendon volume.

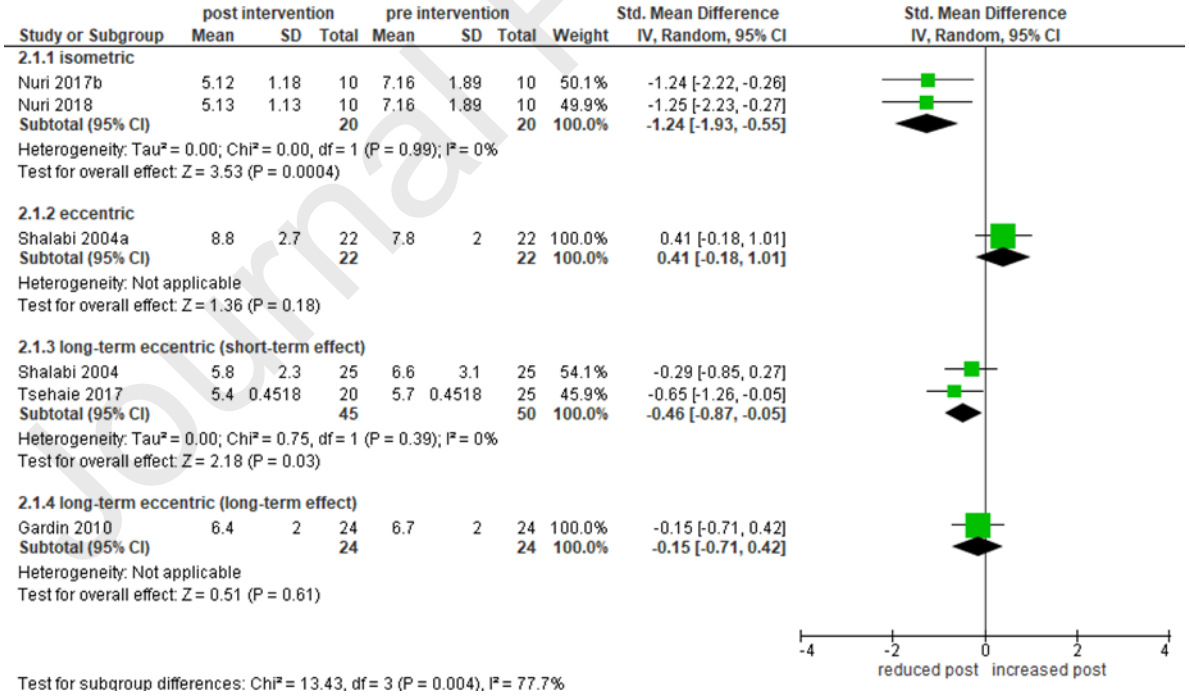

Fig. 3. Forest plot for the meta-analysis of the immediate, short-term and long-term effects of different interventions on midportion Achilles tendinopathy volume.

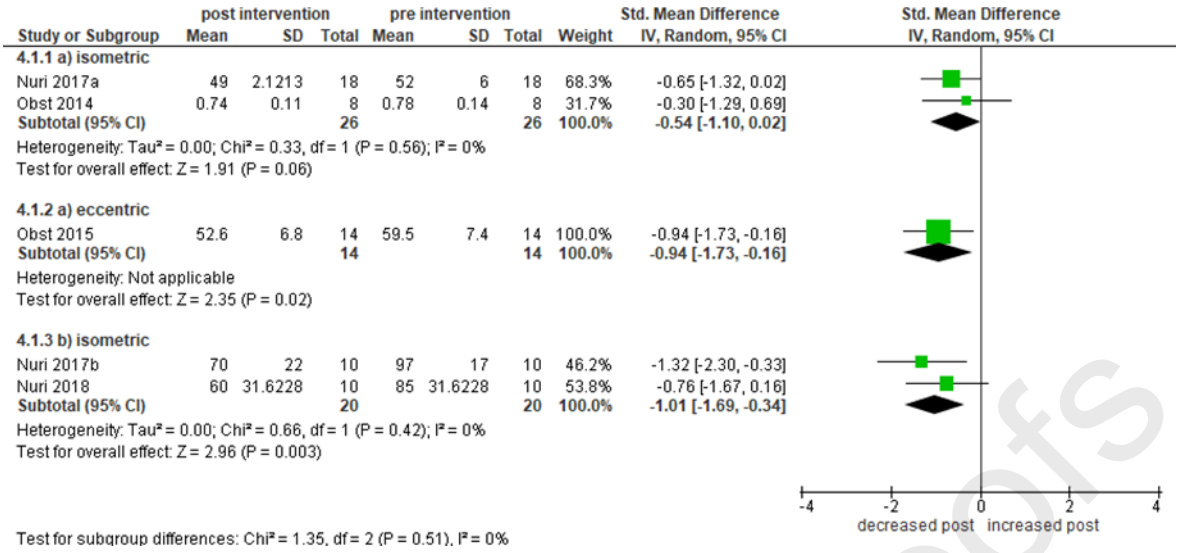

Fig. 4. Forest plot for the meta-analysis of the immediate changes of cross-sectional area in a) healthy people and, b) people with midportion Achilles tendinopathy**.**

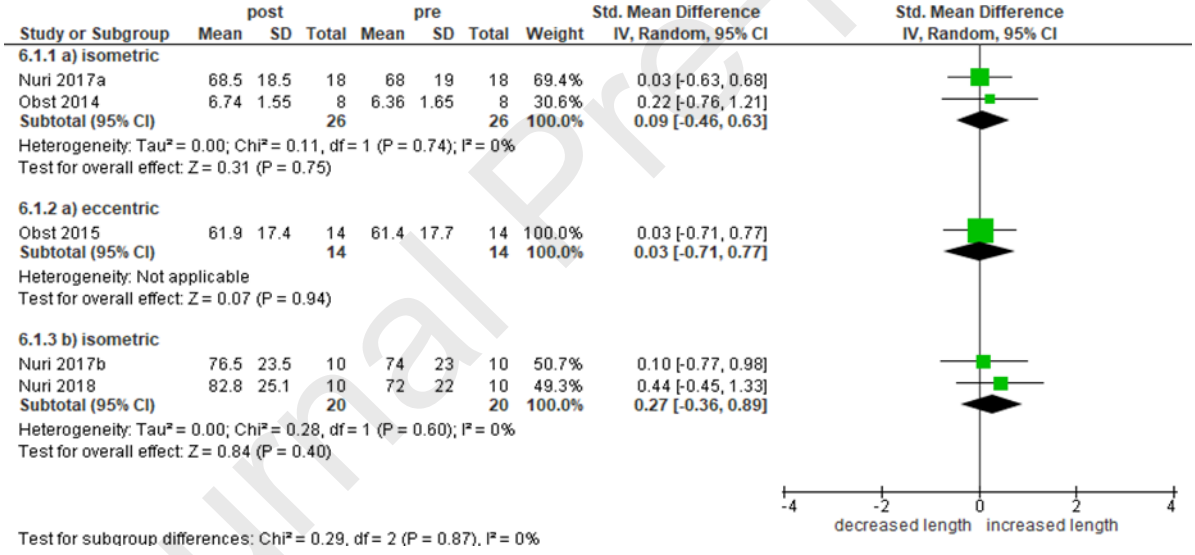

Fig. 5. Forest plot for the meta-analysis of the immediate changes in free Achilles tendon length in a) healthy people and, b) people with midportion Achilles tendinopathy.

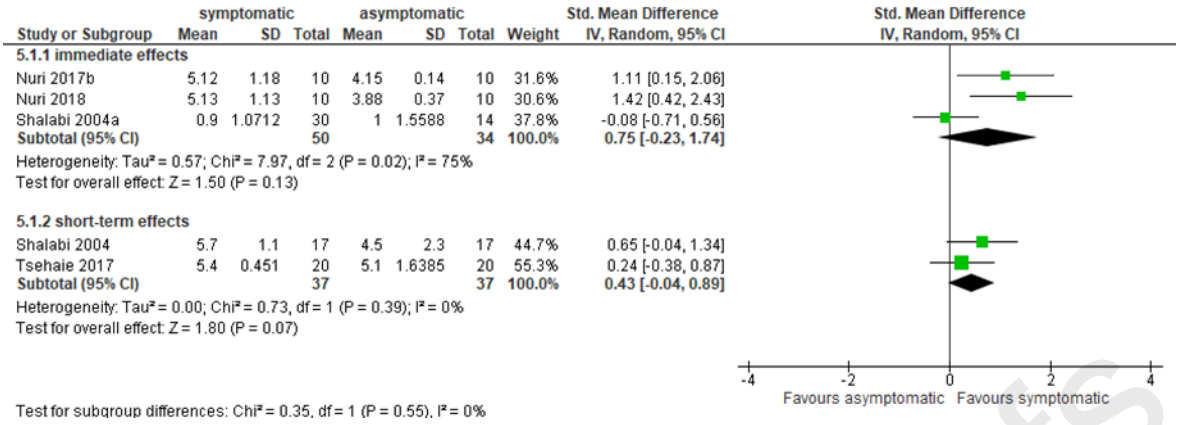

Fig. 6. Forest plot for the meta-analysis of the immediate and short-term effect of different interventions on volume among symptomatic and asymptomatic Achilles tendon.